REMARKS

Applicants have studied the Office Action, and have cancelled all claims and presented new claims in response thereto. It is respectfully submitted that the application, as amended, is in condition for allowance. Prior to entry of the present amendment, claims 46-85 were pending in the present application. Claims 46-85 have been cancelled by virtue of the present amendment, and new claims 86-123 have been added. No new matter has been added. Reconsideration and allowance of the claims in view of the foregoing amendment and the ensuing remarks are respectfully requested.

New claims 86-96 are substantively similar to cancelled claims 46-49, 64-69 and 84-85, but are specifically directed to the treatment of small intestinal bacterial overgrowth. Support for these claims may be found throughout the specification, for example at page 40, lines 4-26, as well as in claims 1 and 4-6 as originally filed in the present application.

New claims 97-104 are substantively identical to cancelled claims 50-55 and 62-63, but are specifically directed to the treatment of small intestinal bacterial overgrowth. Support for these claims may be found throughout the specification, for example at pages 40-43, as well as in claims 7-12 and 19-20 as originally filed in the present application.

New claims 105-115 are substantively similar to cancelled claims 46-49, 64-69 and 84-85, but are specifically directed to the treatment of irritable bowel syndrome. Support for these claims may be found throughout the specification, for example at page 40, lines 4-26, page 33, lines 1-12, as well as in claims 1 and 4-6 as originally filed in the present application.

New claims 116-123 are substantively identical to cancelled claims 50-55 and 62-63, but are specifically directed to the treatment of irritable bowel syndrome. Support for these claims may be found throughout the specification, for example at pages 40-43, as well as in claims 7-12 and 19-20 as originally filed in the present application.

In the Office Action, the Examiner provisionally rejected claims 46-85 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claim 12 of co-pending and co-assigned Patent Application No. 09/374,142. It is respectfully submitted that this rejection has been rendered moot by the cancellation of previously-pending claims 46-85. It is

further submitted that any future obviousness-type double patenting rejection with respect to Patent Application No. 09/374,142 has been obviated by the filing of a Terminal Disclaimer under 37 CFR § 1.321(c), which is attached hereto. The attached Terminal Disclaimer is filed without any admission whatsoever on the part of the Applicants or their Assignee that the newly-presented claims are susceptible to a double patenting rejection over any commonly-owned co-pending Application or issued Patent.

In the Office Action, the Examiner rejected claims 46-85 under 35 U.S.C. § 112, first paragraph, "*because the specification, while being enabling for treatment of SIBO in irritable bowel syndrome patients with a predigested nutritional formula VIVONEX® alone, does not reasonable provide enablement for treatment of SIBO or other SIBO caused conditions by deprivation of all/some nutrients or single nutrient nor of combination therapies with enzymes, absorption alterations, or any other therapies.*" Applicants respectfully submit that this rejection has been rendered moot by virtue of the cancellation of claims 46-85 in the present amendment. However, Applicants were mindful of this rejection when drafting their new claims, which are directed to the treatment of specific conditions, such as small intestinal bacterial overgrowth and irritable bowel syndrome, and offer the following remarks in support of the allowability of the newly presented claims.

In the Office Action, the Examiner stated that the instant application is enabled for treatment of small intestinal bacterial overgrowth in irritable bowel syndrome patients with the use of VIVONEX®, but that it is not enabled for treatment of small intestinal bacterial overgrowth by deprivation of all or some nutrients. As support, the Examiner cited the In re Wands "undue experimentation" factors and, in particular, argued that the nature of the invention (i.e., treating small intestinal bacterial overgrowth by depriving the underlying bacteria of essential nutrients) is not well known, that little direction or guidance is provided in the specification and that a large quantity of experimentation would be required to fulfill the scope of the claims.

As an initial matter, Applicants respectfully submit that the enablement of newly presented claims 113 to 115 is directly supported by the Examiner's comments. Claims 113 to 115 are directed

to treatment of irritable bowel syndrome with VIVONEX®, which is precisely the method of treatment for which the Examiner acknowledges that the application is enabled. Thus, while the Applicants respectfully submit that all of the newly presented claims are enabled, there should be no doubt as to the enablement of claims 113 to 115. Furthermore, VIVONEX® is merely representative of "total enteral nutrition" (TEN) or "elemental" diets, as indicated in the specification at page 40, lines 17-26. Thus, while Applicants have chosen to provide a specific example of nutrient deprivation using VIVONEX® (Example 11, page 73), their choice to present one embodiment in greater detail than others does not defeat the fact that the application is enabled for treatment of small intestinal bacterial overgrowth and/or irritable bowel syndrome by nutrient deprivation of bacterial overgrowth with a broad class of elemental diets. Thus, claims 86-96 and 105-112 are, necessarily, enabled.

Applicants respectfully submit that all of the newly presented claims meet the enablement standard of 35 U.S.C. § 112, first paragraph. The newly presented claims are directed to methods of treating small intestinal bacterial overgrowth and irritable bowel syndrome by nutrient deprivation. While Applicants certainly maintain that these are novel methods of treating the foregoing conditions, it is submitted that considerable guidance is provided for practicing the novel methods presented. Undue experimentation is not required because the specification teaches one of skill in the art how to practice the claimed methods by stating what forms of nutrient deprivation may be used to treat the recited conditions and, in addition, recites the mode of action for the nutrient deprivation methods. For example, on page 40, lines 4-26, the specification teaches that small intestinal bacterial overgrowth and small intestinal bacterial overgrowth-caused conditions (of which irritable bowel syndrome is one) can be treated through nutritional deprivation by feeding an afflicted subject a diet that comprises partially predigested nutrients. A diet of partially predigested nutrients is readily absorbed in the upper gastrointestinal tract, thus starving the bacterial populations which make up small intestinal bacterial overgrowth and which are concentrated in the lower gastrointestinal tract. Total enteral nutrition diets such as VIVONEX® are representative of diets of partially predigested nutrients, as discussed above and clearly indicated on page 40, lines 17-21. Thus, while it is true that the application is enabled for treatment of small intestinal bacterial

overgrowth and associated conditions with VIVONEX®, the application is also enabled for treatment of the same conditions with a broader group of partially predigested nutrient diets (such as total enteral nutrition diets) that are widely known to those of skill in the art.

The enablement standard does not require the Applicants to demonstrate that the claimed invention in fact works; actual reduction to practice is not necessary prior to filing. Gould v. Quigg, 822 F.2d 1074, 1078 (Fed. Cir. 1987); MPEP § 2164.02. "As long as the specification discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of the claims, then the enablement requirement of 35 U.S.C. § 112 is satisfied." In re Fisher, 427 F.2d 833, 839 (CCPA 1970); MPEP § 2164.01(b).

Here, the specification clearly discloses that the claimed methods may be practiced by detecting the presence of small intestinal bacterial overgrowth (either alone, or in a subject with irritable bowel syndrome), and by causing the afflicted subject to consume a diet of partially predigested nutrients to at least partially eradicate the bacterial overgrowth. The claimed methods, far from requiring undue experimentation, are actually quite simple to test, and can certainly be practiced by one of skill in the highly specialized medical arts of treating the recited conditions. Merely by way of example, pages 34 and 35 of the specification teach that known methods of detecting bacterial overgrowth may be practiced in a patient that may or may not be afflicted with irritable bowel syndrome. Page 40 of the specification then teaches that the bacterial overgrowth may be at least partially eradicated by the administration of a diet of partially predigested nutrients, thereby treating the bacterial overgrowth and, in one embodiment, also treating irritable bowel syndrome. Thus, a practitioner of the relevant medical art would not need to engage in undue experimentation to test the methods recited in the newly presented claims. Instead, such a practitioner could carry out well-known steps and observe whether the condition of interest had thereby been improved. Proper guidance is provided for practicing the newly presented claims since the specification clearly teaches the use of a diet of predigested nutrients to eradicate small intestinal bacterial overgrowth and associated irritable bowel syndrome. It is respectfully submitted that the Examiner's rejection of claims 46-85 because the application is not enabled for treatment of bacterial overgrowth or associated irritable bowel syndrome by *single* nutrient deprivation is rendered moot by the cancellation of claims 46-85 and the presentation of claims 86-107, which are directed to

treatment of the conditions by *multiple nutrient deprivation* and which are clearly enabled. However, Applicants do not concede by the presentation of the present amendment that claims 46-85 were not enabled.

The Examiner has also cited a lack of direction of guidance for practicing the claimed methods, stating that only one example of the claimed nutrient deprivation methods is provided. Applicants acknowledge that this component of the Examiner's rejections may be directed to the Examiner's view that methods incorporating *single* nutrient deprivation are not enabled, a rejection that is rendered moot by the presentation of new claims 86-107. However, to the extent that the Examiner's concerns relate to all of the recited methods, Applicants respectfully point out that compliance with the enablement requirement of 35 U.S.C. § 112 does not require the disclosure of working examples. As noted above, actual reduction to practice is not required for an application to be enabled. Accordingly, the Gould court held that "[t]he mere fact that something has not been previously done clearly is not, in itself, a sufficient basis for rejecting all applications purporting to disclose how to do it. Gould, 822 F.2d 1078; MPEP § 2164.02. Thus, the lack of working examples cannot be used as an independent basis for rejecting a claim on enablement grounds; indeed, an invention is enabled if it is otherwise disclosed in a manner allowing one skilled in the art to practice it without undue experimentation. In re Borkowski, 422 F.2d 904, 908 (CCPA 1970); MPEP § 2164.02. In the instant application, a lack of working examples should not form a basis for rejection because, as shown above, the invention is disclosed in a manner well within the purview of one skilled in the highly specialized medical arts of treating the various conditions.

Furthermore, Example 11, found on pages 73 and 74, clearly demonstrates eradication of small intestinal bacterial overgrowth and improvement of irritable bowel syndrome symptoms by practicing one of the claimed methods; the recited conditions are treated by causing the afflicted subjects to ingest a diet comprising VIVONEX®, which is one example of a diet comprising partially predigested nutrients. Thus, Applicants have actually exceeded the requirements of 35 U.S.C. § 112 by providing an *actual* example, even though Applicants respectfully maintain that even working examples are not necessary for the enablement of the presently claimed invention.

It is true that some experimentation may be required to practice the present invention, although the amount of experimentation, as demonstrated above, would be minimal for one skilled in

the art. However, experimentation is permissible and is not a bar to enablement. Wands itself holds that even "a considerable amount of experimentation is permissible, if it is merely routine." In re Wands, 858 F.2d 731, 737 (Fed. Cir. 1988); MPEP § 2164.01. While Applicants maintain that only minimal experimentation would be required to test the presently claimed invention, it is undoubtedly the case that no matter how much experimentation is required, it can only be viewed as routine for one skilled in the relevant art. A medical practitioner would only need to practice a known method of testing for small intestinal bacterial overgrowth and then practice a known method for administering diet comprising partially predigested nutrients, followed by a simple observation of whether a patient's symptoms of one of the recited conditions had improved.

To the extent that the Examiner is concerned about the enablement of newly presented claims 97-104 and 116-123, Applicants respectfully point the Examiner to numerous locations in the specification where guidance is provided such that one skilled in the art could practice the claimed methods. For example at page 40, lines 27-32 and page 41, lines 1-10, clear guidance is provided for nutrient deprivation of bacterial overgrowth through the use of a pancreatic enzyme. Use of the enzyme starves the bacterial overgrowth by digesting nutrients before they reach the distal gut. In addition, pages 41-48 of the specification provide exhaustive guidance for practicing nutrient deprivation of bacterial overgrowth by enhancing digestion and/or absorption of nutrients in the upper gastrointestinal tract. Enhanced digestion in the upper gastrointestinal tract limits the quantity of nutrients that reach the distal gut, thus starving the bacterial overgrowth. In addition, Examples 12 (pages 74-78) and 14 (pages 79-94) provide actual experimental results wherein absorption of nutrients in the upper gastrointestinal tract was enhanced through the use of, *inter alia*, lipids and, thus, bacterial overgrowth in the study subjects was deprived of nutrients.

The Examiner has not specifically argued that the relevant art is unpredictable in nature, but has stated that the treatment of small intestinal bacterial overgrowth by nutrient deprivation is relatively unknown. However, it is respectfully submitted that several recent publications have demonstrated that nutrient deprivation is an effective treatment for small intestinal bacterial overgrowth and that treatment of small intestinal bacterial overgrowth can improve symptoms of irritable bowel syndrome. For example, the Applicants have published several articles demonstrating treatment of small intestinal bacterial overgrowth by nutrient deprivation. E.g., Pimentel, Mark et

al., "*A 14-Day Elemental Diet is Highly Effective in Normalizing the Lactulose Breath Test*," Digestive Diseases and Sciences, Vol. 49, No. 1 (January 2004), pp.73-77 (Exhibit A). The Applicants have also demonstrated an association between treatment of small intestinal bacterial overgrowth and improvement in irritable bowel syndrome symptoms. Pimentel, Mark et al., "*Normalization of Lactulose Breath Testing Correlates with Symptom Improvement in Irritable Bowel Syndrome: A Double-Blind, Randomized, Placebo-Controlled Study*," American Journal of Gastroenterology, Vol. 98, No. 2 (2003), pp.412-419 (Exhibit B). Thus, Applicants respectfully submit that the art is, in fact, predictable from the perspective of one skilled in the art.

In sum, the present application constitutes more than a mere invitation to experiment. Considerable guidance is provided such that one skilled in the art could easily practice the methods recited in newly presented claims 86-107. Such a conclusion is supported by the actual example provided and by the cited passages of the specification.

Applicants believe that the foregoing amendments place the application in condition for allowance, and a favorable action is respectfully requested. If for any reason Examiner finds the application other than in condition for allowance, the Examiner is requested to call the undersigned attorney at the Los Angeles telephone number (213) 488-7100 to discuss the steps necessary for placing the application in condition for allowance should Examiner believe that such a telephone conference would advance prosecution of the application.

Respectfully submitted,
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A 14-Day Elemental Diet Is Highly Effective in Normalizing the Lactulose Breath Test

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Treatment of small intestinal bacterial overgrowth is frustrated by the low efficacy of antibiotics. Elemental diets have been shown to reduce enteric flora. In this study, we evaluate the ability of an elemental diet to normalize the lactulose breath test (LBT) in IBS subjects with abnormal breath test findings. Consecutive subjects with IBS and abnormal LBT suggesting the presence of bacterial overgrowth underwent a 2-week exclusive elemental diet. The diet consisted of Vivonex Plus (Novartis Nutrition Corp., Minneapolis, MN) in a quantity based on individual caloric requirement. On day 15 (prior to solid food), subjects returned for a follow-up breath test and those with an abnormal LBT were continued on the diet for an additional 7 days. The ability of an elemental diet to normalize the LBT was determined for days 15 and 21. A chart review was then conducted to evaluate any clinical benefit 1 month later. Of the 93 subjects available for analysis, 74 (80%) had a normal LBT on day 15 of the elemental diet. When those who continued to day 21 were included, five additional patients normalized the breath test (85%). On chart review, subjects who successfully normalized their breath test had a 66.4 ± 36.1% improvement in bowel symptoms, compared to 11.9 ± 22.0% in those who failed to normalize ($P < 0.001$). An elemental diet is highly effective in normalizing an abnormal LBT in IBS subjects, with a concomitant improvement in clinical symptoms.

KEY WORDS: bacterial overgrowth; enteral nutrition; elemental diet; irritable bowel syndrome.

Bacterial overgrowth is a condition whereby the bacteria of the normally colonized colon are now also colonizing the relatively sterile small intestine. The resulting displacement of bacteria into the small bowel produces a constellation of symptoms including altered bowel habits, abdominal pain, bloating, gas, and distention (1). Classically, bacterial overgrowth is observed in subjects with altered bowel anatomy (2–8). However, recent data suggest that the majority of subjects with IBS may also have an abnormal lactulose breath test (LBT) to suggest bacterial overgrowth in the absence of underlying bowel disease (9, 10). Although there remains some argument as to whether the abnormal LBT in IBS represents accelerated transit or abnormal small bowel flora, IBS symptoms respond to a normalization of the breath test with antibiotics and the LBT abnormality in IBS is significantly different from controls (10).

One major problem in the management of bacterial overgrowth is the poor success of antibiotics in eliminating the large variety of organisms present. In fact, recent studies show that, at best, norfloxacin and ampicillin-clavulanate have a 30 and 50% success in normalizing the LBT to indicate eradication of bacterial overgrowth, respectively (11). This and other experiences lead clinicians to use rotating antibiotics, prolonged courses, and repeated treatment that potentially lead to bacterial resistance. In the case of IBS, neomycin is successful in normalizing the LBT in only 20% of subjects receiving

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the therapy (10). Since IBS is present in more than 10% of the population as a whole (12), widespread antibiotic use in this condition is not wise. As such, a better therapy is needed for bacterial overgrowth and IBS subjects with abnormal LBT that is more reliable and avoids excessive antibiotic use.

Elemental enteral formulations are believed to be entirely absorbed within the first few feet of small intestine. As such, there is a potential for this diet to limit the nutrients for more distally located bacteria of the small intestine. Early research suggests that elemental formulae are capable of reducing stool microbes (13–15). The basis for this change in flora is postulated to be due to nutrient deprivation.

Based on the potential effect of this diet, we hypothesize that a prolonged elemental diet may normalize the LBT finding in IBS subjects and perhaps even supercede antibiotics in treatment efficacy.

METHODS

Patient Population. This was a retrospective review of consecutive cases where elemental diet was used to attempt normalization of LBT in subjects with IBS and abnormal LBT. From 2001 to 2002, subjects who were diagnosed with IBS based on Rome I criteria underwent a LBT. Most subjects found to have an abnormal LBT were then given a trial of antibiotic therapy. After failed attempts to normalize the LBT with antibiotics, subjects were presented the option of the elemental diet since, in preliminary experiences, we had observed normalization of LBT as well as a dramatic clinical response with this therapy.

Elemental Diet. Subjects agreeing to undergo the diet met with the GI motility nurse at Cedars-Sinai Medical Center (T.C.). Based on the height and weight of each subject, the caloric requirement was calculated using the Harris–Benedict equation (16). Subjects then purchased Vivonex Plus (Novartis Nutrition Corp., Minneapolis, MN) in the predetermined quantity. Flavor packets were also used as desired. Patients were all given instructions to dissolve the powdered packets of Vivonex Plus into 250 ml of warm water, add ice, and blend for 1 min. In general, subjects were asked to create three meals a day by dividing the daily recommendations into three equal servings.

Subjects then took the elemental formulation for a period of 14 days. During that time, no food ingestion was allowed including gum, candy, soft drinks (diet or otherwise), and caffeine. Any prescription medications were continued throughout the course.

On day 15, subjects returned for a follow-up LBT. If the LBT was still abnormal on day 15, subjects were encouraged to persist with the diet for an additional 7 days, followed by another LBT. No patient was asked to continue beyond 21 days.

Lactulose Breath Testing. For the LBT, subjects were asked to fast from 7 PM the night before the test. They were also asked to avoid heavy proteins or legumes in the previous evening's meal. On the day of testing, subjects presented to the GI Motility Lab at Cedars-Sinai Medical Center. After an initial breath sample was collected, subjects were asked to ingest a syrup containing 10 g of lactulose (Inalco Spa, Milano, Italy, packaged by Xactdose Inc., South Beloit, IL). After ingestion, breath samples were taken every 15 min until 180 min had elapsed. All breath samples were alveolar and analyzed using a Model SC Quintron Gas Chromatograph (Quintron Instrument Co., Milwaukee, WI). This was used to determine the concentration of hydrogen and methane in breath samples after correction for alveolar quality using the CO_2 concentration in the breath sample. Values for hydrogen and methane were plotted graphically over time. A normal breath test was defined as one that exhibited a rise of breath hydrogen or methane never >20 ppm with the rise beyond 90 min after the ingestion of lactulose (11). All breath tests were coded and randomized. A blinded reader (M.P.) interpreted all results as normal or abnormal. The success of the elemental diet was then determined.

Clinical Outcomes. As part of their clinical care, patients completing the elemental diet were encouraged to return for a follow-up outpatient evaluation at 1 month after completion. Although not initially designed to be a prospective study, during the clinic visit, an attempt was made to quantify any improvement quantitatively (percentage improvement) or qualitatively. A retrospective chart review was conducted on these subjects to define the clinical outcomes in their IBS symptoms from this treatment.

Other specific information was also collected from the chart review. This information included identifying the response among IBS subgroups and identifying the reason for using vivonex, and an attempt was made to determine why any failure of the elemental diet might have taken place.

Statistical Analysis. The proportion of subjects normalizing their LBT was evaluated at day 15. The additional benefit of continuing the elemental diet for an additional 7 days among those who did not normalize at day 15 was also evaluated. The LBT profile was compared before and after the elemental diet, with each time point being compared using a t-test. Data are expressed as mean ± SE.

RESULTS

Patient Population. At the time of review, 124 IBS subjects with an abnormal LBT had agreed to try the elemental diet. Of these subjects, 14 were excluded from analysis, as they could not tolerate the diet and dropped out before completion of the 14 days. In 15 other subjects, the day 15 breath test could not be found. Some of these subjects included out-of-city/state referred patients who did not have access to convenient breath testing. Two other patients had only the day 15 breath test and not the initial breath test. Since the initial abnormal breath test could not be confirmed, these too were excluded. This left 93 subjects for analysis.

Success of the Elemental Diet. By day 15, 74 of the 93 (80%) subjects receiving the elemental diet normalized their LBT. Of the 19 subjects who did not normalize the LBT by day 15, data were available for 17 subjects on day 21. Of these 17 subjects, only 5 additional subjects normalized their LBT with the extended course. The combined success rate for the 14-day and extended course of elemental diet was then 79 of 93 subjects (85%).

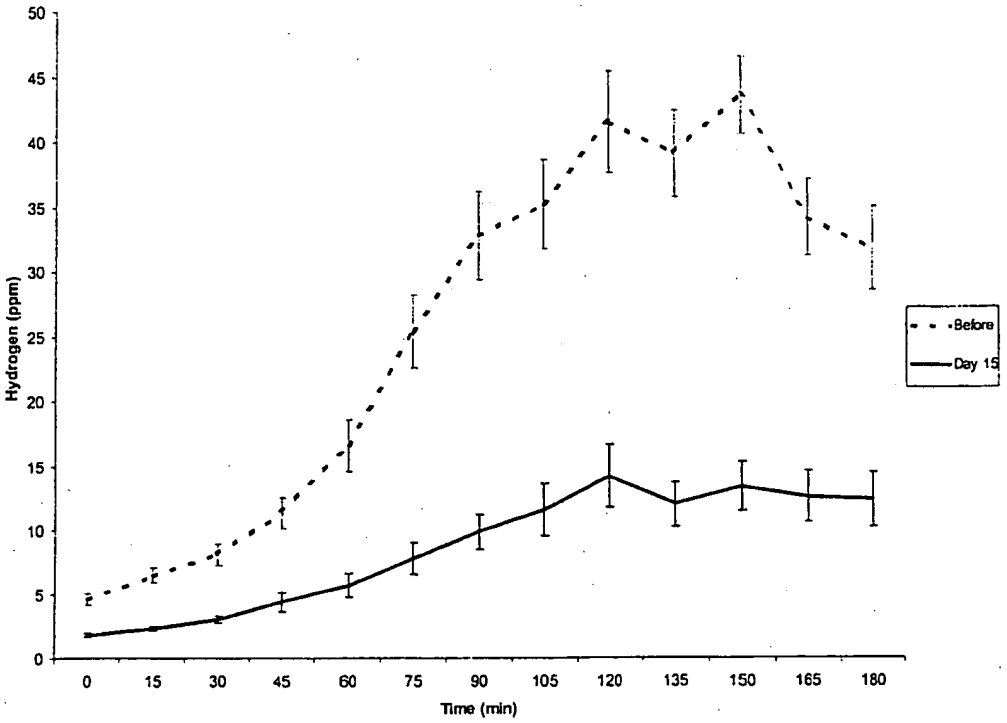

Fig 1. Comparison of lactulose breath test profiles before and after 14 days of Vivonex Plus.

When the actual LBT plot of hydrogen was compared before and after 14 days of elemental diet, all time points were significantly improved among the 93 subjects (Figure 1).

Clinical Outcomes. As far as clinical outcomes were concerned, of 93 subjects, 63 returned for their 1-month follow-up. Of the 63 subjects undergoing elemental diet with clinical follow-up, 13 chose to use the diet without any attempt at antiobiotics. The remaining 50 subjects had failed antibiotic treatment as a reason for choosing the diet.

Among the 63 returning for follow-up, 52 (83%) successfully eradicated. This rate of eradication was identical to that of the whole group, indicating that failure to eradicate was not the reason subjects did not return for follow-up.

Of 63 subjects, quantitative improvement was documented in the chart for 36 subjects, and of these 36 subjects, 28 had documented normalization of LBT with elemental diet. The percentage improvement in IBS symptoms among those who normalized was $66.4 \pm 36.1\%$, compared to $11.9 \pm 22.0\%$ in those who failed to normalize ($P < 0.001$).

On evaluating IBS by subgroup among the 52 subjects with successful eradication after elemental diet, 14 were diarrhea predominant, 25 were alternators, 12 had constipation-predominant IBS, and 1 had only bloating as the main concern. Of the 14 diarrhea-predominant subjects, 12 reported having regular bowel movements at 1-month follow-up and 1 still had diarrhea (1 had no indication of status). In the group of alternators, 18 had regular bowel movements after the diet, 4 said they felt better, 1 felt the same, and 2 were not sure. Among the 12 constipation predominant, 9 were regular, 2 continued to have constipation, and 1 was not sure.

On trying to identify the reason for failure of the elemental diet, subjects who failed to eradicate their overgrowth ($n = 11$) were reviewed. At the time of the review, three of these subjects had confirmed or suspected inflammatory bowel disease, one has since been found to have colonic inertia, one had adhesions from a previous surgery, and one was noncompliant with the elemental diet. Any possible explanation for failed therapy in the remaining five was unknown.

DISCUSSION

To our knowledge this is the first successful use of a short-term elemental diet in normalizing the LBT for the treatment of bacterial overgrowth. Vivonex Plus is able to normalize a preexisting abnormal LBT in 80% of subjects after 14 days of therapy. This benefit is far greater than that seen for antibiotics (10, 11). Furthermore, this normalization of LBT translated into a clinical benefit.

Elemental formulations have been available for more than two decades. An elemental diet formulation consists of hydrolyzed nutrients such that digestion of the product by the recipient will be minimal. The obvious application of this type of formulation is in subjects with a compromised or foreshortened digestive system where formal digestion is compromised. In the 1980s and early 1990s, there was interest in the use of elemental diet for subjects with Crohn's disease (17). Although the results were mixed, data seemed to suggest a benefit, and in some studies, an elemental diet fared as well as steroids in inducing remission (18–20). The mechanism of this beneficial effect was perceived to be related to "bowel rest" rather than the nutritional reconstitution of the subject (21). Interestingly, most contemporary theories of IBD demand the presence of lumenal bacteria as a necessary antigenic stimulation without which there would be little or no inflammation in these disorders. The effect we saw in the normalization of LBT and suspected elimination of small intestinal bacteria may suggest a bacterial mechanism by which elemental diets positively influence Crohn's disease.

In the case of bacterial overgrowth, the hypothesized mechanism for the use of an elemental diet is based on the rapid absorption and assimilation of the elemental formulation. If it is quickly absorbed, there may be little available substrate for the bacteria, which we expect is more distally located in the small intestine of IBS subjects. This explanation may, however, be overly simplistic. Three other explanations are possible with some support from the literature. It is well recognized that bile and its contents have some influence on small bowel bacteria. Bile has stimulatory effects on phase III of the migrating motor complex (22). Phase III, otherwise known as the "intestinal housekeeper" is a cycling wavefront responsible for cleansing the small bowel between meals (23). The absence of phase III is known to result in small intestinal bacterial colonization (24–27). Two studies demonstrate that the ingestion of an elemental diet can produce a dramatic increase in CCK and consequently gallbladder emptying (28, 29). Therefore, one might hypothesize that the increased bilious fluid due to elemental formula ingestion would stimulate phase III, leading to a reduction in small bowel flora.

A second possible mechanism for bacterial suppression with this diet may relate to the mucosal immune system. Data suggest that this same diet can accentuate jejunal secretion of immunoglobulins (30). In the study by Colombel et al., 20 min after jejunal perfusion of an elemental diet, lumenal IgG, IgA, and albumin were significantly increased. Based on this, it is possible that the immune effects of elemental diet are beneficial in clearing the small bowel of organisms.

Finally, elemental formulations may directly affect bacteria of the GI tract. Early observations of the effect of an elemental diet on stool microflora show that this diet significantly reduces coliforms, enterococci, and bacteroides (13). The same study showed that the longer subjects were on the diet, the lower the bacterial counts. Other studies have confirmed these data (14, 15, 31, 32). Even more interesting is that, even when the duodenum is colonized with bacteria, an elemental diet can reduce or eliminate the organisms (31). Since one would expect the duodenum to be exposed to the nutrient composition of the elemental feeding, nutrient deprivation would not explain the reduction of duodenal flora seen. Perhaps the composition of the elemental formula itself has inhibiting effects on bacteria.

Another important point from this study is the continued finding that normalization of the LBT determines the outcome of IBS symptoms. This is consistent with the previous findings by our group (10). The current paper also shows that all subgroups of IBS have a benefit, including the diarrhea, constipation, and alternating subgroups.

In conclusion, an elemental diet is capable of normalizing an abnormal LBT in a much greater proportion of patients than antibiotics. The mechanism of this effect is unclear but likely is not as simple as nutrient deprivation of the bacterial organisms. This treatment appears also to be a viable alternative to antibiotics. In addition, the diet has clinical benefits as evidenced by 1-month follow-up which depends on its ability to normalize the LBT.

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Normalization of Lactulose Breath Testing Correlates With Symptom Improvement in Irritable Bowel Syndrome: A Double-Blind, Randomized, Placebo-Controlled Study

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OBJECTIVE: We have recently found an association between abnormal lactulose breath test (LBT) findings and irritable bowel syndrome (IBS). The current study was designed to test the effect of antibiotic treatment for IBS in a double-blind fashion.

METHODS: Consecutive IBS subjects underwent an LBT with the results blinded. All subjects were subsequently randomized into two treatment groups (neomycin or placebo). The prevalence of abnormal LBT was compared with a gender-matched control group. Seven days after completion of treatment, subjects returned for repeat LBT. A symptom questionnaire was administered on both days.

RESULTS: After exclusion criteria were met, 111 IBS subjects (55 neomycin, 56 placebo) entered the study, with 84% having an abnormal LBT, compared with 20% in healthy controls ($p < 0.01$). In an intention-to-treat analysis of all 111 subjects, neomycin resulted in a 35.0% improvement in a composite score, compared with 11.4% for placebo ($p < 0.05$). Additionally, patients reported a percent bowel normalization of 35.3% after neomycin, compared with 13.9% for placebo ($p < 0.001$). There was a graded response to treatment, such that the best outcome was observed if neomycin was successful in normalizing the LBT (75% improvement) (one-way ANOVA, $p < 0.0001$). LBT gas production was associated with IBS subgroup, such that methane excretion was 100% associated with constipation-predominant IBS. Methane excretors had a mean constipation severity of 4.1, compared with 2.3 in all other subjects ($p < 0.001$).

CONCLUSIONS: An abnormal LBT is common in subjects with IBS. Normalization of LBT with neomycin leads to a significant reduction in IBS symptoms. The type of gas seen on LBT is also associated with IBS subgroup. (Am J Gastroenterol 2003;98:412–419. © 2003 by Am. Coll. of Gastroenterology)

INTRODUCTION

Irritable bowel syndrome (IBS) is a common GI disorder, seen in more than 15% of the population (1, 2). Despite this high prevalence and much research interest, the cause of IBS remains unknown.

Over the last few years, progress has been made in characterizing IBS. Studies have demonstrated altered gut motility (3), peripheral (4) and central (5) sensory dysfunction, as well as an exaggerated response to stress (6) in this syndrome. However, there is no finding that can be identified in a majority of patients, and by extension there is no diagnostic test that is associated with IBS. As a result, investigators have created complex diagnostic schema, such as the Rome criteria, to help diagnose and categorize the syndrome (7, 8).

One consistent clinical finding in IBS is gas in combination with bloating and visible distention (9, 10). Koide *et al.* recently found small intestinal gas to be significantly increased in IBS patients compared with control subjects (11), regardless of whether subjects conform to diarrhea, constipation, or pain subgroups.

Excessive small intestinal gas can occur as a result of increased production of gas within the gut by bacterial fermentation. A condition known to produce excessive small bowel gas is small intestinal bacterial overgrowth (SIBO). We recently reported that 78% of subjects with IBS have a positive lactulose breath test (LBT), suggesting the presence of SIBO (12). Although provocative, this study had some intrinsic design issues, and the data do not account for how an abnormal breath test might produce the diarrhea and constipation in IBS. Recent data suggest that children with encopresis have excessive breath methane on LBT (13). This finding has not been extended to adults with constipation-predominant IBS.

In this double-blind, randomized, placebo-controlled study, we test whether an abnormal LBT is more prevalent in IBS subjects than in normal control subjects, whether antibiotic treatment in IBS leads to an improvement in

symptoms, and whether this is based on antibiotic-induced normalization of breath test. Secondly, LBT profiles are evaluated to determine if gaseous constituents vary between IBS subgroups.

MATERIALS AND METHODS

Study Population

Study subjects were recruited by advertisements in local newspapers, radio, and IBS support groups throughout the greater Los Angeles area. To avoid referral bias, subjects were not recruited through the GI motility clinic or any gastroenterology practice based at Cedars-Sinai Medical Center. Subjects were included if they met Rome I criteria for IBS (7). Rome I was chosen because it does not prejudice between diarrhea and constipation, and no peer-reviewed publications were available to validate Rome II as a diagnostic strategy (14). Subjects were excluded if they had taken antibiotics within the previous 3 months, had a previous lactulose breath test (LBT), or a history of diabetes, thyroid disease, intestinal surgery (except cholecystectomy or appendectomy), connective tissue disease, narcotic use, or known GI disease. Subjects with renal insufficiency, hearing impairment, probiotic use, or allergy to aminoglycosides were also excluded. Approval from the institutional review board and written, informed consent from the participating subjects were obtained.

In an initial comparison, 15 gender-matched normal controls were identified based on the absence of all Rome I criteria. These subjects underwent lactulose breath testing, and the prevalence of abnormal breath test was compared with subjects with IBS.

Study Design

Subjects presented to the GI Motility Laboratory having fasted from 7:00 PM the night before. They were instructed not to ingest legumes or a heavy meal for dinner the night before evaluation. Good oral hygiene was recommended, and smoking was not permitted on the day of testing.

Before the LBT, subjects completed a symptom questionnaire asking them to grade nine IBS symptoms (abdominal pain, diarrhea, constipation, bloating, sense of incomplete evacuation, straining, urgency, mucus, and gas) on a severity score of 0–5, as has been previously used and recommended (15–17). All questions were answered based on subjects' recall of the preceding 7 days (17).

Subjects then underwent an LBT by ingesting 10 g of lactulose (Inalco, Milano, Italy; packaged by Xactdose, South Beloit, IL) followed by 1–2 ounces of water after an initial baseline breath sample. Breath samples were then collected at 15-min intervals for 180 min. End expiratory breath samples were taken to ensure alveolar gas sampling. This was achieved via a 750-ml gas collection bag (Quintron Instrument, Milwaukee, WI). Samples were analyzed for hydrogen, methane, and carbon dioxide using a Model SC, Quintron gas chromatograph (Quintron Instrument). Carbon dioxide measurements were used to correct for the quality of alveolar sampling. Measurements were plotted graphically as previously described (12). Patients and investigators were blinded to the result of the breath test.

All subjects were randomized by personnel not associated with the study to receive, in a double-blind fashion, either neomycin (500 mg) (Teva Pharmaceuticals USA, Sellersville, PA) or matching placebo *b.i.d.* for 10 days. Seven days after completion of the antibiotic or placebo, subjects returned for a repeat questionnaire and LBT. A 7-day follow-up was chosen because in our experience the abnormal breath test in IBS can recur as early as 2 wk after antibiotic normalization. As part of the follow-up questionnaire, subjects were asked to subjectively rate the amount of improvement they experienced as a percent normalization of bowel function, and they again rated the perceived severity of the nine bowel symptoms described earlier. Compliance was assessed by pill count. To comply with institutional review board requirements, follow-up LBT results could not be blinded, so patients could seek appropriate medical therapy for their test result.

At the completion of enrollment, all initial and follow-up breath tests were coded and randomized by personnel not involved in the interpretation of the test. A blinded reviewer (M.P.) interpreted the results and was asked to categorize the breath tests based on whether the test met the criteria for normal LBT. A normal LBT was defined as no rise of breath hydrogen (H_2) or methane (CH_4) concentration before 90 min of lactulose, with a definitive rise never more than 20 ppm during 180 min of measurement (18–21). Studies that fell out of this range were categorized as abnormal. A second set of criteria for breath test interpretation was also used, whereby the traditional two peaks to suggest bacterial overgrowth were required. Because the two peak method was not as well validated a technique (19) as the ppm method, this finding was only used to compare the prevalence of this finding with healthy controls.

Measures of Outcome

Data were analyzed using an intention-to-treat method. The primary outcome measure was based on a composite score (CS) calculated from the three main IBS symptoms (abdominal pain, diarrhea, and constipation, each on a scale of 0–5) to generate a score out of 15 (most severe). This was done to account for the severity of all potential IBS subgroups. Because other IBS symptoms (such as straining) would worsen or improve depending on whether patients started with diarrhea or constipation, respectively, minor criteria were not included in the CS. In addition, because reduction in colonic organisms could result in an improvement in gas and bloating, irrespective of bacterial overgrowth, gaseous symptoms too were excluded from the score. The percent improvement in the CS was then compared between placebo and neomycin. In addition, the overall percent bowel normalization as determined by patient reporting was likewise compared.

The prevalence of a true clinical response was then determined and compared between placebo and neomycin. A true clinical response was defined as a ≥50% reduction in CS. Secondarily, a true clinical response was also assessed based on subjects reporting their overall percent bowel normalization. A ≥50% normalization implied a true clinical response. This method of analysis closely followed the multinational consensus recommended guidelines for data analysis in IBS clinical studies (16).

Secondary endpoints included a similar analysis of gender subgroups. Subsequently, IBS subgroups were identified, whereby diarrhea-predominant IBS was deemed present when diarrhea severity (0–5 scale) was greater than constipation in any individual subject. The opposite proportion determined constipation predominance. This means of identifying diarrhea- and constipation-predominant subgroups was chosen because criteria for these subgroups are not validated and are based subjectively on physician interview (14). This approach further reduced bias because subjects would not be aware of the interest in subgrouping their predominant feature.

A post hoc analysis was then conducted on all abnormal breath test results to determine if the type of gas produced on LBT was related to IBS subgroup. The abnormal breath tests were divided into two abnormal test groups: hydrogen production only and any methane production. The relationship between constipation-predominant IBS and diarrhea-predominant IBS to the type of gas seen was determined. Subsequently, in a more objective fashion, the severity score for diarrhea and constipation were then compared between gas types. Finally, a score based on the difference between constipation and diarrhea severity (C-D) was determined. The C-D score was used to examine the relative weight of constipation to diarrhea in individual subjects (the more positive the score the greater was the dominance of constipation compared with diarrhea). Subjects with identical score for constipation and diarrhea severity were excluded from these analyses. This C-D score was also compared between gas types.

Finally, to support the principal that the abnormal test in IBS was not due to rapid transit, the mean breath test profile in constipation- and diarrhea-predominant IBS was compared. Because it is suggested in the literature that diarrhea-predominant IBS is associated with rapid transit (22–24) and constipation predominant IBS with slow transit (22, 23), the hydrogen profile should be different in both groups.

Statistical Analysis

The number of subjects enrolled in the study was determined based on the detection of a 10% difference between placebo and neomycin. This further assumed a 15% variance and an $\alpha = 0.05$ with power of 90% in a two-sided analysis.

Quantitative data were compared using the Student's *t* test, with results expressed as mean ± SE. Comparisons of qualitative data used the Fisher exact test for comparison of IBS subjects with healthy controls. All other qualitative data comparisons used the χ^2 test. A one-way analysis of variance (ANOVA) was used to compare the results of the three groups: placebo-treated, neomycin with unsuccessful normalization of LBT, and neomycin-treated with successful normalization of LBT.

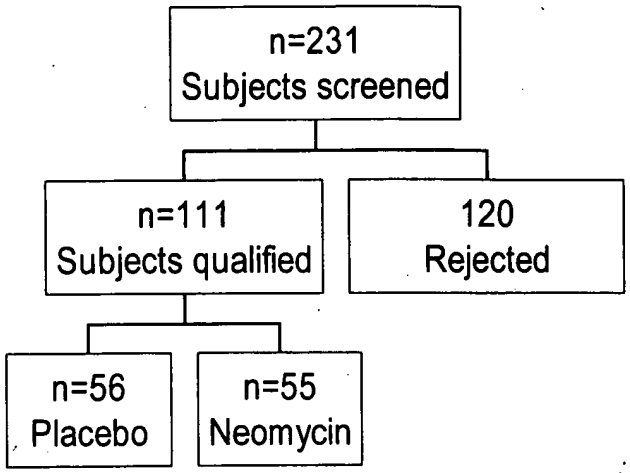

Figure 1. Patient flow chart.

RESULTS

Subject Demographics

A total of 231 subjects were screened (Fig. 1). Of these, 111 met enrollment criteria. However, 10 of these 111 subjects had incomplete data (six in neomycin group and four in placebo group). The specific reasons for incomplete data were voluntary premature withdrawal (n = 3), no follow-up breath test (n = 4), failure to return for follow-up (n = 1), no follow-up questionnaire (n = 1), and premature withdrawal by subject due to severe diarrhea (n = 1). Despite the incomplete data, these subjects were included in the intention-to-treat analyses and counted as no (0%) improvement. The baseline characteristics were similar for the neomycin and placebo groups (Table 1).

Table 1. Comparison of Demographics Between Placebo and Neomycin Groups

Characteristic	Placebo	Neomycin	*p* Value
n	56	55	
Age (yr)	41.9 ± 0.2	44.7 ± 0.2	ns
Gender (female/male)	27/29	34/21	ns
Baseline composite score	8.7 ± 0.4	8.8 ± 0.3	ns
Abnormal breath test	47 (84)	46 (84)	ns
Diarrhea-predominant IBS	21 (40)*	25 (48)†	ns
Constipation-predominant IBS	20 (38)*	18 (35)†	ns
Other IBS subgroup	11 (21)*	7 (13)†	ns

Data are mean ± SE or n (%). Baseline composite score = pain severity + diarrhea score + constipation score (each on a scale of 0–5) before treatment. Other IBS subgroup = subjects with constipation severity = diarrhea severity.

* Only 52 subjects in the placebo group completed the questionnaire sufficiently to determine this result.

† Only 52 subjects in the neomycin group completed the questionnaire sufficiently to determine this result.

Case-Control Comparison

IBS subjects had a higher prevalence of abnormal LBT than gender-matched controls, with 93 of 111 (84%) subjects fulfilling these criteria, compared with three of 15 (20%) gender-matched controls (OR = 26.2, CI = 4.7–103.9, $p < 0.00001$). When comparing the prevalence of abnormal LBT with double peak, 55 of 111 IBS subjects (50%) were positive, compared with two of 15 healthy controls (13%) ($p = 0.01$).

Primary Outcome Measures

In the intention-to-treat analysis, neomycin resulted in a 35.0 ± 5.0% reduction in CS, compared with a 11.4 ± 9.3% reduction in the placebo group ($p < 0.05$). In the subgroup of patients with abnormal baseline LBT (n = 93), neomycin produced a 35.4 ± 5.6% reduction in CS, compared with a 3.7 ± 10.6% reduction in the placebo group ($p < 0.01$). No difference was seen in subjects with a normal baseline breath test, although a higher placebo rate was reported in this very small group (51%).

Of the 111 subjects, 91 completed their percent bowel normalization question after treatment. Of these 91 subjects, neomycin resulted in a 40.1 ± 5.3% reported bowel normalization, compared with 15.1 ± 3.6% for placebo ($p < 0.001$). Among the subgroup of subjects with abnormal initial breath tests, neomycin resulted in a 44.8 ± 5.6% normalization, compared with 11.0 ± 3.3% for placebo ($p < 0.00001$).

Neomycin was more likely to result in a true clinical response than placebo. Among all subjects receiving neomycin, 24 of 55 (43%) experienced a ≥50% improvement in CS, compared with 13 of 56 (23%) in the placebo group (OR = 4.3, CI = 1.05–6.3, $p < 0.05$). In the subgroup of subjects with abnormal breath tests, 21 of 46 (46%) receiving neomycin had a clinical response, compared with seven of 47 (15%) in the placebo group (OR = 4.8, CI = 1.62–14.7, $p < 0.01$). Using patients' subjective report of percent bowel normalization, in the whole group of subjects who answered this question (n = 91), 50% of subjects receiving neomycin had a true clinical response, in contrast to 17% of subjects getting placebo (OR = 4.8, CI = 1.7–14.4, $p < 0.01$). In those with abnormal initial breath test, 55% of neomycin- and 11% of placebo-treated subjects had a true clinical response (OR = 9.6, CI = 2.5–39.7, $p < 0.0001$). Finally, seven of the eight subjects (88%) who had a normal follow-up LBT after neomycin reported more than 50% normalization of bowel function.

Of the 111 subjects, only the 101 subjects with complete data were used in the remainder of the analyses.

Of 84 out of 101 subjects with an abnormal baseline LBT, 41 were treated with neomycin. Of those 41, eight (20%) achieved normalization of LBT. One of 43 subjects in the placebo group went from an abnormal breath test to normal. A significant difference in symptom response was seen, depending on the outcome of treatment in these abnormal subjects. Specifically, the percent reduction in CS was different in the following three groups: subjects receiving placebo (4.1 ± 11.7%), neomycin-treated group that did not achieve LBT normalization (34.4 ± 6.2%), and neomycin-treated group with LBT normalization (61.7 ± 9.4%) ($p = 0.01$, one-way ANOVA) (Fig. 2). Using patients' self-report of percent bowel normalization, the three groups were more different. Subjects receiving placebo reported 11.0 ± 3.7% normalization, subjects receiving neomycin but not successful normalization of LBT, 36.7 ± 6.1%, and those subjects with normal follow-up LBT after neomycin reported 75.0 ± 6.4% bowel normalization ($p < 0.0000001$, one-way ANOVA).

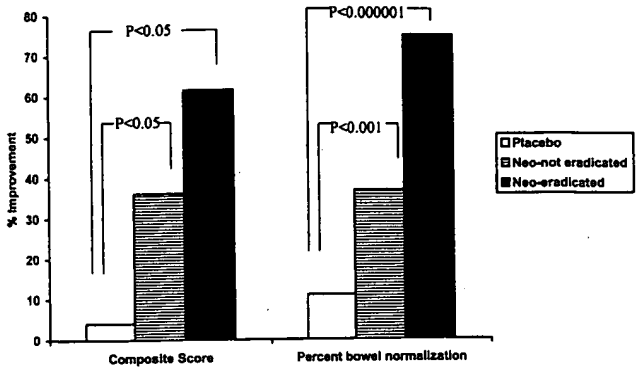

Figure 2. Percent improvement in composite score based on treatment and success in normalizing the LBT. Data = mean percent reduction in composite score; the difference in the composite score was significant ($p = 0.01$, one-way ANOVA). The difference in patient-reported improvement was also significant ($p < 0.000001$, one-way ANOVA). In the neomycin (Neo)-treated groups, the data were analyzed according to success of treatment.

Effect of Gender

Both male and female neomycin-treated subjects were noted to have a significantly greater improvement in percent bowel normalization over those receiving placebo (Fig. 3). Furthermore, there was no difference in response rate between male and female patients.

Type of Gas and IBS Subgroup

The type of gas produced by IBS subjects on LBT was predictive of their subtype of IBS among the 84 subjects with abnormal baseline. After exclusion of subjects with no

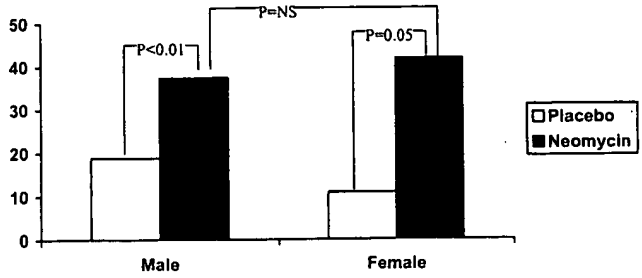

Figure 3. Comparison of percent reported bowel normalization between and within gender groups.

Table 2. Comparison of IBS Subgroups Based on Methane and Hydrogen Excretion With Abnormal Breath Test

	Hydrogen	Methane
Diarrhea (n)	34	0
Constipation (n)	19	12

Total n = 65, after exclusion of subjects with no gas production (n = 4), normal breath test (n = 17), and subjects in whom diarrhea severity = constipation severity (*i.e.*, neither predominant) (n = 15). $p < 0.001$ between groups.

gas production (n = 4) and subjects in whom constipation severity was equal to diarrhea (n = 15), 34 diarrhea-predominant and 31 constipation-predominant IBS subjects were analyzed. Of 31 constipation-predominant subjects, 12 (39%) excreted methane, whereas no methane excretion was seen in the 34 diarrhea-predominant subjects (OR = ∞, CI = 3.7–4.3, $p < 0.001$, positive predictive value = 100%) (Table 2). The severity of constipation was 4.1 ± 0.3 in subjects with methane excretion but only 2.3 ± 0.2 in nonmethane excretors ($p < 0.01$) (Table 3). In a similar comparison, the C-D score was 2.8 ± 0.5 in methane excretors and −0.7 ± 0.3 for hydrogen excretors ($p < 0.00001$) (Table 3).

Transit Comparison

When the mean hydrogen breath test profile was compared between diarrhea- and constipation-predominant IBS subjects, there was no evidence that diarrhea predominance had earlier hydrogen appearance (Fig. 4). In fact, diarrhea and constipation profiles were both virtually superimposable and not different at any time point, with a mean of >20 ppm at 90 min in both groups.

Adverse Events

One subject developed profuse watery diarrhea while taking placebo. The cause of the diarrhea was later found to be food poisoning. Two of the enrolled subjects were found to have other diagnoses. The first subject had an 8-cm mass in the abdomen. The surgical specimen demonstrated non-Hodgkin's lymphoma. This subject was in the placebo group. The second subject was noted to have urinary retention, which precipitated bowel complaints. The second subject was in the neomycin group. Both these subjects had a normal initial LBT. Both were included as part of the intention-to-treat analysis.

Table 3. Evaluation of the Severity of Constipation or Diarrhea Based on Methane Production on Baseline Breath Test

	No Methane	Methane	*p* Value
Constipation severity	2.3 ± 0.2	4.1 ± 0.3	<0.001
Diarrhea severity	3.0 ± 0.2	1.4 ± 0.4	<0.001
C-D score*	−0.7 ± 0.3	2.8 ± 0.5	<0.00001

* C-D score represents the difference between severity of constipation and diarrhea. This was done to show an increased relative weight of constipation to diarrhea with methane excretors.

DISCUSSION

In this double-blind, randomized, placebo-controlled study, we found a higher prevalence of abnormal LBTs in IBS subjects than in controls. In addition, we found that antibiotics were more effective than placebo in terms of symptom improvement. Normalization of the breath test produced an even greater improvement of IBS symptoms, substantiating results from a previous study (12). Furthermore, methane excretion on breath testing was highly associated with the constipation-predominant subgroup of IBS. These findings continue to support an association between abnormal LBT and IBS.

The main question regarding the LBT findings in IBS is its meaning. A simple explanation could be that it represents bacterial overgrowth or an increased number of enteric organisms. Critics argue that the abnormal LBT might be due to rapid transit. Without bacterial culture, the exact explanation is difficult to isolate.

A modest attempt has been made in this study to answer the question of transit. Studies have suggested that small bowel transit is accelerated in diarrhea-predominant IBS (22–24). Similar studies suggest that subjects with constipation-predominant IBS have delayed transit (22, 23). If transit is the explanation for the abnormal breath test findings, then subjects with constipation-predominant IBS should have delayed gas rise on breath test compared with subjects with diarrhea. On the contrary, in our study, breath tests were abnormal irrespective of subgroup of IBS, suggesting that transit alone cannot explain the findings. Figure 4 shows that constipation- and diarrhea-predominant IBS subjects have identical LBT profiles, with early excessive hydrogen production in both. In addition, the transit argument cannot explain the clinical improvement that depends on the normalization of the LBT.

Regarding the explanation of altered intestinal flora in IBS, there is some support in the literature. Fully one third of subjects with acute bacterial gastroenteritis have persistent IBS-like symptoms 6 months after recovering from their acute illness (25). However, no model of sustained microbial challenge is available to explain the chronic symptoms of IBS. Our finding of a high prevalence of abnormal LBT suggests that SIBO might be the persistent antigenic challenge in IBS.

There is also support for the association between altered breath test results and enteric flora in IBS. In one study, 56% of diarrhea-predominant IBS subjects were found to have a positive ^{14}C-xylose breath test (26). In another study, Flagyl was reported to be superior to placebo in reducing clinical symptoms in IBS (27). The authors of that article were uncertain of the mechanism for this improvement.

In a recent study, we showed that a large percent of IBS subjects may have SIBO as diagnosed by LBT (12). Despite some skepticism about the reliability of LBT to diagnose SIBO, there are similarities between SIBO and IBS. Bloating, a feature of SIBO, is also classically associated with

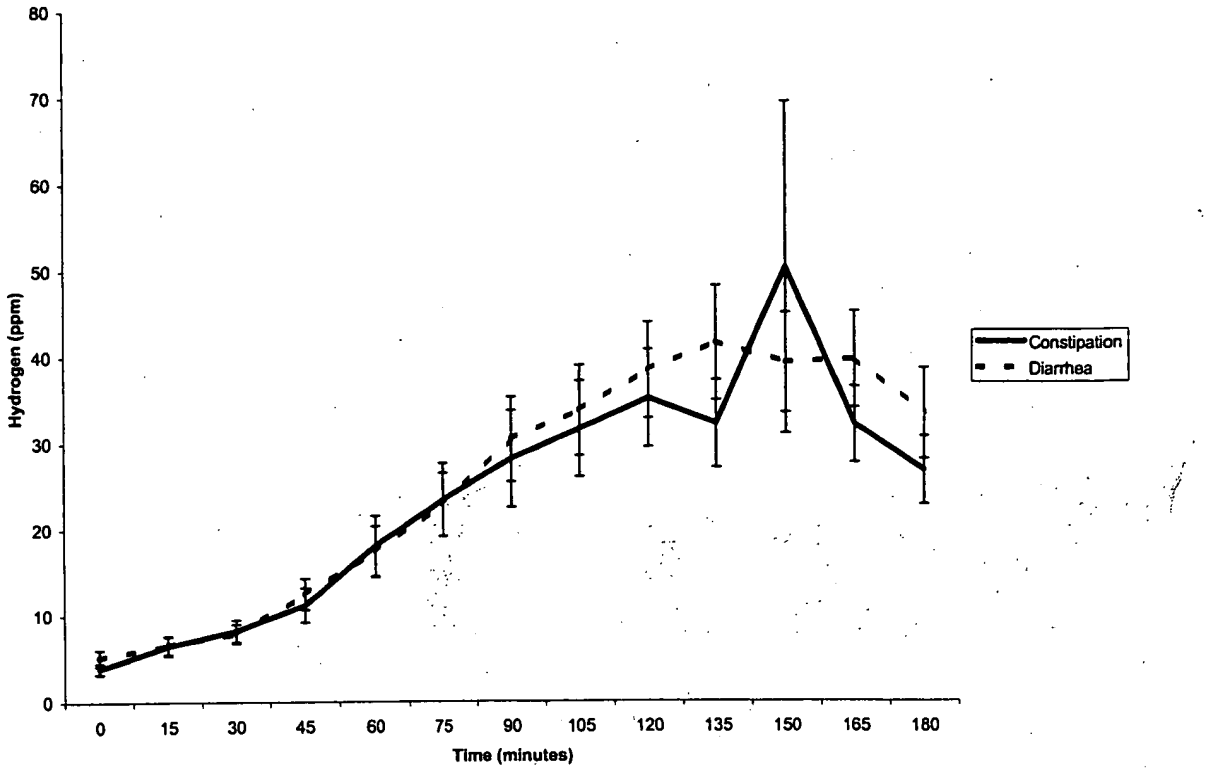

Figure 4. Comparison of the breath hydrogen profiles in diarrhea- and constipation-predominant IBS subjects. No difference was seen at any time point between the constipation- and diarrhea-predominant IBS breath test profiles.

IBS (10). In SIBO, bloating is due to small intestinal fermentation of nutrients. Until recently, gas studies in IBS have been limited to the investigation of flatus. Yet even these studies suggest the presence of excessive bacteria in IBS. King *et al.* found the production of hydrogen by IBS subjects to be elevated fivefold, implying excessive enteric bacteria (28). Recently, data suggest that IBS patients have excessive gas and that this gas is localized to the small intestine (11). One explanation for excessive small bowel gas might be SIBO.

To further substantiate abnormal enteric flora as a contributing cause of IBS, it is necessary to account for the physiological changes seen in IBS. Certainly, bacteria-triggered immune responses can produce many of the classic physiological changes in IBS (29). However, the contrasting diarrhea- and constipation-predominant subgroups in IBS remain unexplained. In our study, we show that methane excretion on breath test has a positive predictive value of 100% for constipation-predominant IBS. This may be another important step in linking bacteria and IBS. There is other literature supporting this idea. Methane is noted to be common in diverticulosis (30) and encopresis (13) and less prevalent in diarrheal conditions, such as Crohn's disease or ulcerative colitis (31–33).

There are some technical limitations with the LBT. First, some groups criticize the reliability of LBT to diagnose SIBO, because in the identification of any infectious process, culture is the gold standard. The main issue with culture is accessibility. Riordan *et al.* compared breath testing with direct culture and found the breath test to lack reliability (34). This and other similar studies were confounded by their selection of subjects who had surgically altered anatomy, predisposing to the development of upper GI tract SIBO. Because SIBO (in surgically naïve patients) is often an expansion of colonic bacteria, the direction of expansion is retrograde, involving first the distal small intestine. As such, direct culture is only practical in the patient whose SIBO is so severe that the bacteria has expanded proximally into the duodenum or proximal jejunum.

Regardless of the argument as to whether the breath test reliably detects SIBO, excessive flora, or rapid transit, the data in our study support a role of the LBT in IBS treatment, as it is only when the subsequent LBT is normal that the greatest symptom improvements are realized.

One difficult-to-explain result of the study is the high placebo response in subjects without abnormal breath test. The most likely possibility is the low number of subjects in the placebo group (n = 9). As a result, the variation was large.

Lastly, an important issue to discuss is antibiotic use in IBS. Neomycin, although statistically more effective than placebo, was only able to normalize the breath test 20% of the time. This may be because of the large numbers and types of enteric organisms (35–38) or bacterial resistance.

Furthermore, the widespread use of antibiotics may not be wise before more effective antibiotics are tested.

In summary, we have shown that a high percentage of IBS subjects have abnormal LBT. Antibiotic treatment results in a significant improvement in IBS symptoms and clinical response rates, and this correlates with normalization of LBT. Furthermore, the LBT appears associated with constipation-predominant IBS when methane is present during testing. Evidence also suggests the abnormal LBT results in IBS are not due to transit alone. Work is needed to characterize the role of methanogenic and nonmethanogenic bacteria in the GI manifestations of this common condition.

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